In Vitro Activities of the β-Lactamase Inhibitors Clavulanic Acid, Sulbactam, and Tazobactam Alone or in Combination with β-Lactams against Epidemiologically Characterized Multidrug-Resistant *Acinetobacter baumannii* Strains

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Acinetobacter baumannii is an important nosocomial pathogen usually in the context of serious underlying disease. Multidrug resistance in these organisms is frequent. The β-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam have intrinsic activity against *Acinetobacter* strains. To evaluate their potential therapeutic usefulness, we determined the in vitro activity of ampicillin, sulbactam, ampicillin-sulbactam, cefoperazone, cefoperazone-sulbactam, piperacillin, piperacillin-sulbactam, tazobactam, piperacillin-tazobactam, amoxicillin, clavulanic acid, amoxicillin-clavulanic acid, ticarcillin, and ticarcillin-clavulanic acid against multidrug-resistant *A. baumannii*. All isolates were epidemiologically characterized by RAPD [random(ly) amplified polymorphic DNA] analysis and/or pulsed-field gel electrophoresis and represented different strain types, including sporadic strains, as well as outbreak-related strains. The MICs were determined by agar dilution on Mueller-Hinton agar (using fixed concentrations, as well as fixed ratios for β-lactamase inhibitors) and the E-test. The majority of E-test results were within two dilutions of those recorded by agar dilution, with the exception of piperacillin-tazobactam. Sulbactam was superior to clavulanic acid and tazobactam and may represent an alternative treatment option for infections due to multiresistant *A. baumannii* strains. β-Lactamase inhibitors have intrinsic activity but do not enhance activity of β-lactams against *A. baumannii*. Testing with the inhibitor added at a fixed concentration as recommended for piperacillin-tazobactam and ticarcillin-clavulanic acid by the National Committee for Clinical Laboratory Standards may falsely suggest high activity or gives uninterpretable results due to trailing. If combinations are used for testing, fixed ratios may give more useful results.

Acinetobacter species are significant opportunistic pathogens that are usually associated with a serious underlying disease. Nosocomial infections and hospital outbreaks have been mainly attributed to *Acinetobacter baumannii* (24). Multidrug resistance is common among these organisms and leaves few therapeutic options. A recent outbreak in New York revealed 12% of *A. baumannii* isolates to be resistant to all standard antimicrobial agents (15). Imipenem is considered the "gold standard" treatment; however, resistance to this agent has been reported (13, 15, 17), mediated through carbapenem-hydrolyzing enzymes or a permeability barrier (2, 6). Alternative therapies are therefore needed. The β-lactamase inhibitors sulbactam and tazobactam have been reported to possess intrinsic antibacterial activity against *Acinetobacter* strains at concentrations achievable in humans (~40 and 5.5 to 51 mg/liter, respectively) (1, 20, 26, 28, 29). Sulbactam combinations are bactericidal against *A. baumannii* in in vivo models (21, 32). Ampicillin plus sulbactam was used successfully in the treatment of serious infections during an outbreak caused by an epidemic *A. baumannii* strain that was susceptible to ampicillin plus sulbactam only but resistant to all other available antimicrobial agents, including imipenem (13).

The testing of antimicrobial agents in combination has been the subject of several publications (25, 27). At issue is the problem of what inhibitor concentration to use. The National Committee for Clinical Laboratory Standards (NCCLS) guidelines for testing of amoxicillin-clavulanate requires a ratio of 2:1, respectively; however, ticarcillin-clavulanate is tested with a fixed inhibitor concentration of 2 mg/liter (19). The German (DIN) guidelines are conducted with a fixed concentration of 2 mg of clavulanate/liter (8), and the British (BSAC) breakpoints are set irrespective of the inhibitor concentration (5). In testing with sulbactam combinations, the NCCLS guidelines require a ratio of β-lactam to sulbactam of 2:1, whereas the German DIN requires a fixed concentration of 8 mg/liter (8) and the BSAC have no recommendations. With piperacillin-tazobactam, both the NCCLS guidelines and the German DIN guidelines require a fixed inhibitor concentration of 4 mg/liter.

The objectives of the present study were to evaluate the activity of three β-lactamase inhibitors alone and in combination with their respective β-lactam components against epidemiologically characterized *A. baumannii* strains and to compare the methodology of sensitivity testing with a fixed concentration of inhibitor versus a ratio of inhibitor to β-lactam. We also compared E-test and agar dilution sensitivity testing of β-lactams and inhibitors.

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TABLE 1. Distributions of antibiotic susceptibilities as determined by agar dilution against β-lactams, β-lactamase inhibitors, and combinations for 115 *A. baumannii* strains

Antimicrobial[a]	No. of isolates for which the MIC (mg/liter) was:														MIC (mg/liter)		No. ND[b]	% Sensitivity[c]
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256	MIC_{50}	MIC_{90}		
AMP								2	3	21	37	11	2	39	32	≥256		4.4
SAM (2:1)						9	32	24	22	8	6	12	2		4	64		75.7
SAM (8)	92		1						1	1	2	1		17	≤0.03	≥256		80.9
SUL				1	7	30	36	16	4	8	11	2			2	32		
CFP			1					2	1	6	25	27	13	40	64	≥256		8.7
CFP-SUL (2:1)				1	1	9	29	30	20	4	8	12	1		4	64		81.7
CFP-SUL (8)	91		2	1								4	5	11	≤0.03	128		82.5
AMX						1		1	6	34	25	8	1	39	32	≥256		7
AMC (2:1)						1	2	6	26	47	14	7	3	9	16	64		30.4
AMC (2)				1	2	1		3	13	34	11	4	4	35	16	≥256	7	18.5
CLA							1	17	29	50	4	3	1	10	16	64		
TIC							3	17	34	18	7	6		30	16	≥256		62.6
TIM (2:1)							6	26	33	24	10	5	3	8	8	64		77.4
TIM (2)			1			3	9	17	23	16	5	6	2	27	16	≥256	6	63.3
PIP								6	20	20	13	15	5	36	32	≥256		40.0
TZP (2:1)						1	2	12	38	32	14	6	2	8	16	64		73.9
TZP (4)	12	2	2		1			4	12	13	6		7	18	16	≥256	38	59.7
TZB							5	20	29	31	16	5	1	8	16	64		
PIP-SUL (2:1)					1	11	33	28	16	4	10	10	1	1	4	32		80.9
PIP-SUL (8)	80												3	16	≤0.03	≥256	16	80.8

[a] The fixed ratio of β-lactam to inhibitor was 2:1. The fixed concentration of β-lactamase inhibitor is given in milligrams per liter. AMP, ampicillin; SAM, ampicillin-sulbactam; SUL, sulbactam; CFP, cefoperazone; AMX, amoxicillin; AMC, amoxicillin-clavulanate; CLA, clavulanic acid; TIC, ticarcillin; TIM, ticarcillin-clavulanate; PIP, piperacillin; TZP, piperacillin-tazobactam; TZB, tazobactam.

[b] No. ND, number of isolates not determinable.

[c] That is the percentage of strains that were sensitive.

MATERIALS AND METHODS

Bacterial strains. *A. baumannii* strains (*n* = 115) were selected from a collection of clinical isolates from Germany, the United States, and various European countries that were obtained between 1991 and 2000 (11, 23, 31). Phenotypic species identification was performed according to the methods of Bouvet and Grimont, including growth at 37, 41, and 44°C; the production of acid from glucose; gelatin hydrolysis; and the use of 14 different carbohydrates (4). To ensure that copy strains were eliminated, strains were selected on the basis of having a unique fingerprint pattern as determined by RAPD [random(ly) amplified polymorphic DNA] analysis and/or pulsed-field gel electrophoresis according to previously described methods (10, 23). Sporadic strains, as well as outbreak-related strains (i.e., one strain per given outbreak), were included.

Susceptibility testing. Standard powders of the following β-lactams and β-lactamase inhibitors were obtained from their respective manufacturers: amoxicillin (GlaxoSmithKline, Munich, Germany), ampicillin (Pfizer, Karlsruhe, Germany), cefoperazone (Pfizer), piperacillin (Wyeth-Lederle, Munich, Germany), ticarcillin (GlaxoSmithKline), clavulanic acid (GlaxoSmithKline), sulbactam (Pfizer), and tazobactam (Wyeth-Lederle). Agar dilution MICs were determined according to published standards and guidelines (19) with cation-adjusted Mueller-Hinton agar (Oxoid, Wesel, Germany) and a final inoculum of 10^4 CFU/ml per spot and with antimicrobial concentrations ranging from 0.03 to 128 mg/liter. MICs were determined with each compound on its own and in combination with β-lactamase inhibitors. Clavulanate was added to amoxicillin and ticarcillin at a fixed concentration of 2 mg/liter and at a β-lactam/inhibitor ratio of 2:1. Sulbactam was added to ampicillin, cefoperazone, and piperacillin at a fixed concentration of 8 mg/liter and a ratio of 2:1. Tazobactam was added to piperacillin at a fixed concentration of 4 mg/liter and a ratio of 2:1. The following NCCLS breakpoints were used for interpretation of the sensitivity of both β-lactams alone and their various inhibitor combinations: amoxicillin and ampicillin at 8 mg/liter and cefoperazone, piperacillin, and ticarcillin at 16 mg/liter.

The MIC for 91 randomly selected strains was also determined on Mueller-Hinton agar by using the E-test (AB Biodisk, Solna, Sweden) according to the recommendations of the manufacturer. The β-lactams amoxicillin, ampicillin, cefoperazone, piperacillin, and ticarcillin were investigated. β-Lactam–β-lactamase inhibitor combinations were investigated either at a fixed ratio or with a fixed concentration of the inhibitor as follows: amoxicillin-clavulanic acid (2:1), ampicillin-sulbactam (2:1), cefoperazone-sulbactam (2:1), piperacillin-tazobactam (4 mg/liter), and ticarcillin-clavulanic acid (2 mg/liter).

RESULTS

The in vitro activities of the three β-lactamase inhibitors alone and in combination with their respective β-lactam components against 115 epidemiologically defined *A. baumannii* strains as determined by agar dilution are shown in Table 1. *A. baumannii* strains were highly resistant to ampicillin, amoxicillin, and cefoperazone, with MICs at which 50% of the isolates are inhibited (MIC_{50}s) of 32 to 64 mg/liter, MIC_{90}s of ≥256 mg/liter, and susceptibility rates of 4.4, 7, and 8.7%, whereas 40 and 62.6% of strains were susceptible to piperacillin (MIC_{50} and MIC_{90} = 32 and ≥256 mg/liter, respectively) and ticarcillin (MIC_{50} and MIC_{90} = 16 and ≥256 mg/liter, respectively).

The β-lactamase inhibitors when tested alone were more active than the β-lactams against most of the isolates, with lower MIC_{50} and MIC_{90} values. Sulbactam (MIC_{50}, 2 mg/liter; MIC_{90}, 32 mg/liter) was more active than tazobactam (16 and 64 mg/liter), and clavulanic acid (16 and 64 mg/liter) (Table 1). Of 115 isolates, 13 (11%) exhibited sulbactam MICs of ≥32 mg/liter, and 9 of these were outbreak-related strains (data not shown). The combination of a β-lactam and a β-lactamase inhibitor had the effect of lowering the MIC in all but the most resistant strains. A ratio of β-lactam to inhibitor gave MIC distributions similar to those of the inhibitors on their own. The addition of sulbactam to ampicillin raised the sensitivity level from 4% to 75.7 to 80.9% depending on whether it was present as a fixed concentration or as a ratio. A similar level of sensitivity, as well as a similar MIC distribution, was found when sulbactam was tested alone or when combined with cefoperazone or piperacillin at a fixed ratio, reflecting the intrinsic activity of the inhibitor (Fig. 1 and 2). A bimodal distribution of MIC can be seen with sulbactam combinations tested at
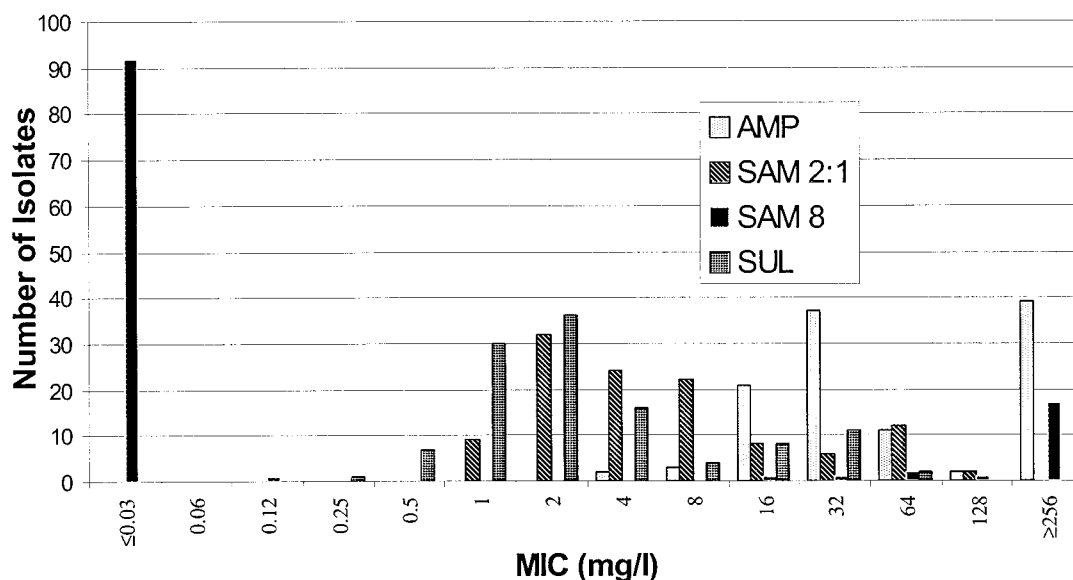
FIG. 1. MIC distribution of ampicillin-sulbactam combinations.

a fixed concentration of inhibitor of 8 mg/liter, with the majority of strains (80%) showing MICs of ≤0.03 mg/liter and some strains showing an MIC of ≥32 mg/liter. A fixed concentration of sulbactam thus demonstrated a lower MIC_{50} but a higher MIC_{90} than was obtained with a ratio of the three β-lactams.

Tazobactam combined with piperacillin increased the level of sensitivity from 40% to 59.7 to 73.9%. However, 38 strains did not record a readable MIC against piperacillin-tazobactam with the fixed inhibitor concentration of 4 mg/liter. Colonies on the agar dilution plates "trailed," i.e., a few colonies were present at a number of concentrations, and no clear endpoint was reached; however, there were not enough colonies to justify recording a high MIC. Most of these strains might be read as susceptible because the corresponding MICs of 35 of them, upon testing with a piperacillin-tazobactam ratio of 2:1, were

≤16 mg/liter. However, MICs would falsely suggest a high activity of the combination if this growth is ignored. When tested alone with tazobactam, 29 of the 38 strains had an MIC of ≤8 mg/liter (range, 2 to 32 mg/liter). Although testing with the fixed concentration of tazobactam gave a lower overall level of susceptibility than a ratio (59.7 versus 73.9%), the fixed tazobactam concentration gave the impression of hypersusceptibility since 16 strains had an MIC of ≤0.12 mg/liter (Fig. 3). A similar observation was also made with the other fixed combinations—amoxicillin-clavulanic acid (2 mg/liter), ticarcillin-clavulanic acid (2 mg/liter), and piperacillin/sulbactam (8 mg/liter)—with 6 to 14% of strains giving unreadable endpoints due to trailing.

Clavulanate was the least effective inhibitor. In combination with amoxicillin, there was 18.5 to 30.4% susceptibility reported, but clavulanate showed greater activity if combined
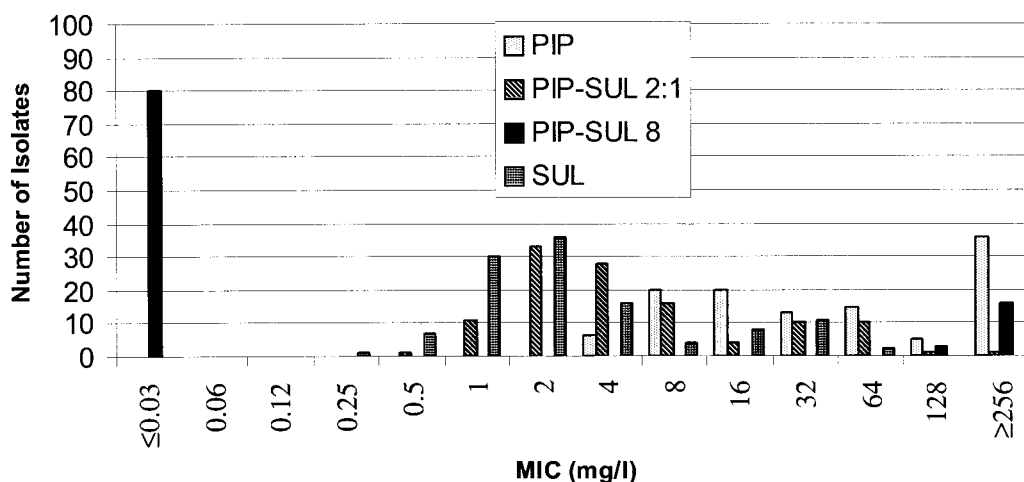
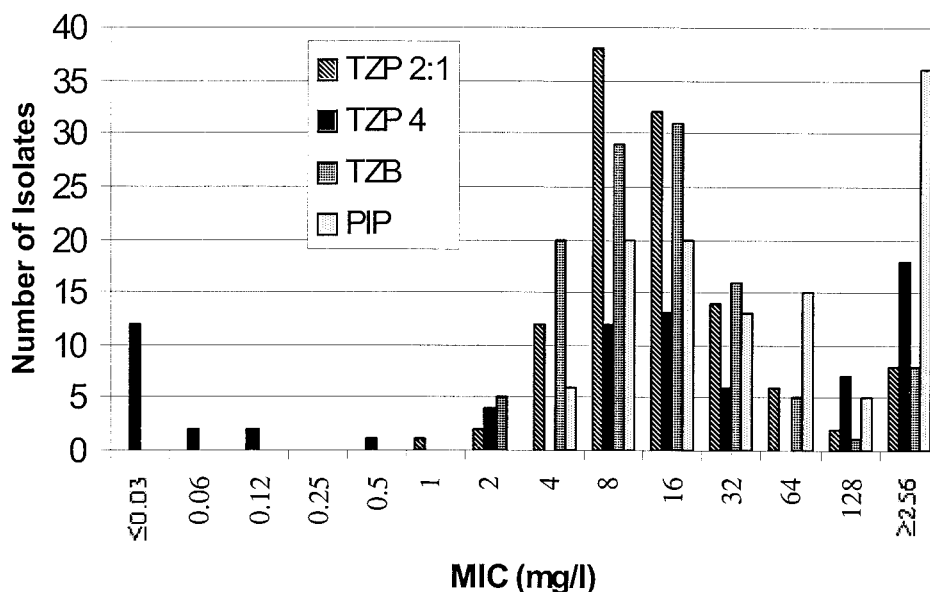FIG. 2. MIC distribution of piperacillin-sulbactam combinations.

FIG. 3. MIC distribution of piperacillin-tazobactam combinations.

with ticarcillin, a result that reflects more the ticarcillin potency than the potency of the combination. A total of 62% of the strains were susceptible to ticarcillin alone, and this value was only raised 0.7% with the addition of 2 mg of clavulanate/liter. A fixed concentration of clavulanate resulted in less sensitivity than was achieved in tests with the ratio; however, these differences were not as pronounced as with the other inhibitor combinations.

The distribution of MICs determined by E-test is shown in Table 2. Compared to agar dilution MIC, E-tests recorded higher sensitivity levels with inhibitor combinations (Table 3). An exception to this were tests with ticarcillin-cefoperazone, which showed less sensitivity. MIC_{50} values as determined by E-test were similar to those obtained by agar dilution. Cefoperazone by E-test recorded an elevated MIC_{50} of ≥ 256 compared to 64 by agar dilution. The piperacillin-tazobactam combination tested by E-test yielded an $MIC_{50} \leq 0.03$ mg/liter

versus an MIC_{50} of 16 mg/liter obtained by agar dilution; however, if the strains that did not record an MIC by agar dilution due to trailing are omitted from the E-test comparison, there was no difference in the MIC_{50} between the two testing methods. These differences may result from the particular diffusion property of tazobactam in the E-test strips, leading to an antimicrobial activity that is not comparable to the conditions of tazobactam dissolved in agar. Of note, the classical E-test ellipse is often not seen with *A. baumannii* and piperacillin-tazobactam, and instead the growth inhibition zone rather resembles a cylindrical shape of uniform inhibition (Fig. 4). The lower panel of Fig. 4 shows a pear-shaped inhibitory zone with ticarcillin-clavulanate. In this case, an elliptical inhibitory zone caused by the ticarcillin meets the cylindrical zone of the clavulanate. In addition, in a few cases the results were unreadable. The E-test also gives the impression of strains hypersusceptible to ticarcillin-clavulanate and pipera-

TABLE 2. Distributions of antibiotic susceptibilities as determined by E-test of β-lactams, β-lactamase inhibitors, and combinations for 91 *A. baumannii* strains

Antimicrobial[a]	No. of isolates for which the MIC (mg/liter) was:													MIC (mg/liter)		No. ND[b]	% Sensitivity[c]
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256	MIC_{50}	MIC_{90}		
AMP								1	3	13	27	6	2	38	64	≥256	1	4.4
SAM (2:1)					12	30	22	7	4	8	2		1		2	16	5	87.2
CFP							1		3	20	10	3		52	>256	≥256	2	4.5
CFP-SUL (2:1)					2	24	24	17	5	6	5			2	2	16	6	91.8
AMX						1		1	4	17	22	12		33	64	≥256	1	6.7
AMC (2:1)						5	11	3	13	21	17	6	1	13	16	≥256	1	35.6
TIC						1	1	3	17	22	15	4		26	32	≥256	2	49.4
TIM (2)	19					3	2	6	17	15	5	5		19	8	≥256		68.1
PIP							1	6	17	19	8	2		36	32	≥256	2	48.3
TZP (4)	45						2	6	7	1	1	2		23	≤0.03	≥256	4	70.1

[a] The fixed ratio of β-lactam to inhibitor was 2:1. The fixed concentration of β-lactamase inhibitor is given in milligrams per liter. AMP, ampicillin; SAM, ampicillin-sulbactam; CFP, cefoperazone; SUL, sulbactam; AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; TIC, ticarcillin; TIM, ticarcillin-clavulanate; PIP, piperacillin; TZP, piperacillin-tazobactam.
[b] See Table 1, footnote *b*.
[c] See Table 1, footnote *c*.

TABLE 3. Agreement between E-test and agar dilution results

Antimicrobial[a]	No. of E-test MICs within an agar dilution MIC of:							No. not determined	% MICs within:	
	≥−3	−2	−1	0	+1	+2	≥+3		1 dilution	2 dilutions
AMP		3	7	60	14	2	4	1	89.0	94.5
SAM (2:1)	11	31	36	8				5	48.4	82.4
CFP	2	2	9	40	12	11	13	2	67.0	81.3
CFP-SUL (2:1)	4	21	29	27	2		2	6	63.7	86.8
AMX		2	6	63	13	5	1	1	90.1	97.8
AMC (2:1)	1	13	12	37	17	5	5	1	72.5	92.3
TIC		1	5	44	33	5	1	2	90.1	96.7
TIM (2)	21	5	6	39	11	3	1	5	61.5	70.3
PIP	2	10	16	36	18	3	4	2	76.9	91.2
TZP (4)	16	14	8	11	4	3	6	29	25.3	44.0

[a] The fixed ratio of β-lactam to inhibitor was 2:1. The fixed concentration of β-lactamase inhibitor is given in milligrams per liter. AMP, ampicillin; SAM, ampicillin-sulbactam; CFP, cefoperazone; SUL, sulbactam; AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; TIC, ticarcillin; TIM, ticarcillin-clavulanate; PIP, piperacillin; TZP, piperacillin-tazobactam.

cillin-tazobactam (MIC, ≤0.03 mg/liter) that were not seen by agar dilution. Table 3 shows the agreement between the E-test and agar dilution methods. The majority of β-lactam MICs were within one dilution on either side of their corresponding agar dilution MIC (90 to 67%). When combined with an inhibitor, this level of agreement is considerably less. Only 25% of piperacillin-tazobactam E-test MICs were within one dilution, and 44% were within two dilutions of the agar dilution MIC.

DISCUSSION

A. baumannii is an important nosocomial pathogen, particularly in intensive care units. A major cause for concern is its increasing multidrug resistance which now encompasses what is considered the drugs of choice, the carbapenems. The SENTRY surveillance in 1997 to 1999 found 11% resistance to the carbapenems (9), highlighting the need for new treatment options to combat this threat. Imipenem-amikacin drug combinations have been tried in a mouse model but did not improve upon imipenem monotherapy (22), although synergy between these drugs has been reported in vitro (18). Another combination, ampicillin-sulbactam, has been shown to be efficacious in treatment of multidrug-resistant *A. baumannii* meningitis and ventilator-associated pneumonia (7, 13, 33).

It has been shown previously that β-lactamase inhibitors, sulbactam in particular, have intrinsic activity against *A. baumannii* (1, 12, 16, 27, 29). The activity of β-lactam–β-lactamase inhibitor combinations used at a fixed ratio of β-lactam to inhibitor is reflected mainly by the MICs of the respective inhibitors alone. For sulbactam this was shown by nearly identical MIC distributions of the three β-lactam–β-lactamase inhibitor combinations and sulbactam alone and also by nearly identical MICs for any given strain (data not shown). Similar results were observed with tazobactam- and clavulanate-containing combinations if compared to the inhibitors alone. We found the use of a fixed concentration of inhibitor against sensitive isolates to record a lower MIC than when tested at a ratio of 2:1. For example, all combinations with sulbactam at a fixed concentration of 8 mg/liter yielded MICs of ≤0.03 mg/liter for nearly 80% of the *A. baumannii* strains. This may falsely suggest excellent potency of the drug combination

(MIC$_{80}$, ≤0.03 mg/liter) against these otherwise β-lactam-resistant strains. In contrast, if used at a ratio with a β-lactam, as proposed by the NCCLS, the corresponding drug combination would have MICs at least six dilution steps higher (MIC$_{80}$, 16 mg/liter) without a major impact on the susceptibility rates. This finding is unsurprising given that sulbactam is more potent than any of the β-lactam agents tested and that at 8 mg/liter the majority of isolates are susceptible (12), a finding confirmed here. However, when tested against intermediate and resistant isolates, a fixed ratio yields a lower MIC$_{90}$. In spite of these considerable MIC differences, there is no difference in the overall resistance levels between fixed-ratio and fixed-concentration testing with sulbactam.

Given the achievable concentrations of sulbactam in serum after parenteral administration of usual doses, this may not have a major impact upon the therapy of respiratory tract or bloodstream infections. However, in the case of meningitis, in which drug levels 10 times the MIC are required in the spinal fluid for cure, it may well make a difference if the MIC of the drug combination is ≤0.03 or 8 mg/liter, and clinical treatment failures have been reported (13). In contrast, Jones and Dudley found with the *Enterobacteriaceae* that testing ampicillin-sulbactam by disk diffusion and agar dilution overestimated clinical resistance, and these authors also proposed a change in MIC breakpoint (14).

A. baumannii β-lactamases are not as sensitive to clavulanate as β-lactamases from other gram-negative organisms such as BRO from *Moraxella catarrhalis* (3) or even not sensitive at all. Ampicillin resistance in *M. catarrhalis* is almost completely abolished with clavulanate, whereas it is not clear if the improved activity of β-lactams such as amoxicillin or ticarcillin in combination with clavulanate compared to the use of β-lactam alone reflects any impact of the inhibitor on the β-lactamase of *A. baumannii*. The intrinsic activity of clavulanate against *A. baumannii* is low, and this is also reflected by the relatively low level of sensitivity when clavulanate is used in combination with amoxicillin. The higher activity of ticarcillin-clavulanate combinations reflects the greater potency of the β-lactam. Testing amoxicillin with a fixed clavulanate concentration leads to an overall lower level of sensitivity in *A. baumannii* compared to testing with a fixed ratio (18% versus
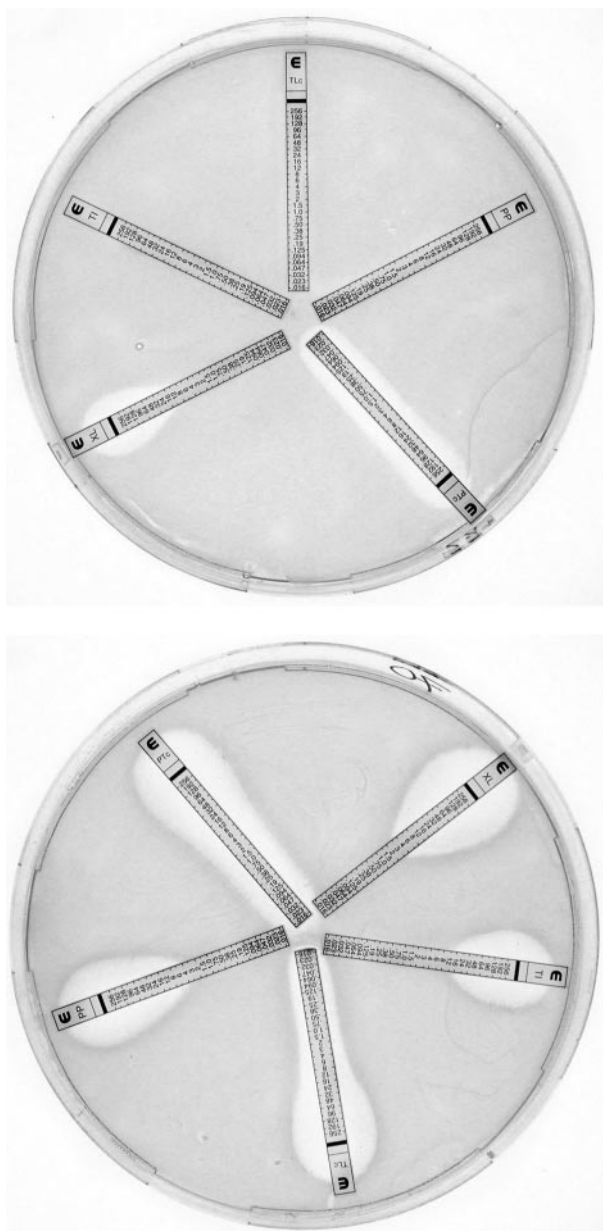
of the different amounts of clavulanate. However, given that <1% of the isolates we tested had a clavulanate MIC of ≤2 mg/liter, testing with a fixed concentration of 2 mg/liter would be expected to have had little effect. We therefore recommend that ticarcillin-clavulanate be tested at a 2:1 ratio for a better indication of sensitivity of *A. baumannii*.

The NCCLS guidelines for sensitivity testing with piperacillin-tazobactam require a fixed concentration of 4 mg of inhibitor/liter. This correlates well with in vivo data in which the C_{max} values approximate 4 mg/liter (14). We tested our strains with a fixed tazobactam concentration and ratio. Our data do not show any difference in MIC_{50} between the testing methods; however, with the fixed concentration, the MIC_{90} is higher. In spite of this, there is a group of strains—those with a tazobactam MIC of ≤4 mg/liter —that appear to be highly sensitive to piperacillin-tazobactam with MICs ranging from ≤0.03 to 0.12 mg/liter. As with sulbactam tested with the fixed concentration, this could result in overestimating the level of susceptibility and may lead to underdosing and treatment failure, especially in the case of meningitis or other deep-seated infections. If a fixed inhibitor concentration is used that is above or close to the MIC of the strain, the interpretation of MIC results is further hampered by the fact that these strains either do not grow, even at the lowest concentration of the β-lactam agent, or that growth for each dilution of the β-lactam is equally reduced to a few colonies that prevent interpretation of the results (trailing). The activity of piperacillin-tazobactam could thus not be determined for 33% of strains by agar dilution. Similar observations were made when we used broth microdilution (unpublished observations).

The use of E-tests for β-lactam testing was in agreement with limits previously reported by Visalli et al. (30). However, testing of β-lactam-inhibitor combinations at fixed concentrations such as piperacillin-tazobactam and ticarcillin-clavulanate by E-test for *A. baumannii* is not to be recommended since the results may overestimate sensitivity. It can be speculated that antimicrobial disk susceptibility testing of β-lactam–inhibitor combinations that also involves fixed inhibitor concentrations can lead to misleading results.

We conclude that in vitro results of β-lactam–β-lactamase inhibitor combinations against *A. baumannii* are mainly determined by the activity of the inhibitors alone and influenced by whether a fixed ratio of inhibitor to β-lactam or a fixed concentration of the inhibitor is used. This can be attributed to the intrinsic activity of the inhibitors and not to β-lactamase inhibition (7). Sulbactam has good intrinsic antimicrobial activity against multidrug-resistant *Acinetobacter* strains at concentrations readily achievable in human serum (29) and may therefore have some therapeutic implications in the treatment of infections caused by multidrug-resistant *A. baumannii* infections. However, *A. baumannii* strains with elevated sulbactam MICs (≥32 mg/liter) exist, and the majority of these strains were outbreak related. Susceptibility testing of this inhibitor alone is therefore clearly warranted. Tazobactam and clavulanic acid, in contrast, were only moderately active or inactive. Antimicrobial susceptibility testing of β-lactam–β-lactamase inhibitor combinations by agar dilution or E-test should be interpreted with caution. This is especially true if the inhibitor is used at a fixed concentration that is at or above the MIC of the inhibitor against the majority of strains. The combinations

FIG. 4. (Top panel) E-test results showing an elliptical zone of inhibition with amoxicillin-clavulanate and the cylindrical inhibition zone of piperacillin-tazobactam in an *A. baumannii* strain resistant to piperacillin. XL, amoxicillin-clavulanate; PTc, piperacillin-tazobactam; TI, ticarcillin; TLc, ticarcillin-clavulanate; PIP, piperacillin. (Bottom panel) Classical elliptical inhibitory zone against amoxicillin-clavulanate, piperacillin, and ticarcillin. Piperacillin-tazobactam shows the cylindrical inhibition, and ticarcillin-clavulanate shows a pear-shaped zone.

30%), a finding previously reported in *Escherichia coli* (25, 27). It has been argued that amoxicillin-clavulanate should be tested like ticarcillin-clavulanate, at a fixed concentration and not at a ratio (27). This is to err on the side of caution and assume strains are less sensitive because a fixed concentration produces a lower level of sensitivity. With ticarcillin-clavulanate the same result is obtained: a ratio produces a higher level of sensitivity than a fixed concentration and is a reflection

do render ampicillin- or piperacillin-resistant *A. baumannii* strains susceptible to the combination, but further clinical studies or animal models of infection are needed to confirm whether these strains respond to standard doses of the drug combinations.

REFERENCES

1. **Acar, J. F., F. W. Goldstein, and M. D. Kitzis.** 1993. Susceptibility survey of piperacillin alone and in the presence of tazobactam. J. Antimicrob. Chemother. **31**(Suppl. A):23–28.
2. **Afzal-Shah, M., N. Woodford, and D. M. Livermore.** 2001. Characterization of OXA-25, OXA-26, and OXA-27, molecular class D beta-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii.* Antimicrob. Agents Chemother. **45**:583–588.
3. **Berk, S. L., and J. H. Kalbfleisch.** 1996. Antibiotic susceptibility patterns of community-acquired respiratory isolates of *Moraxella catarrhalis* in Western Europe and in the USA. J. Antimicrob. Chemother. **38**:85–96.
4. **Bouvet, P. J. M., and P. A. D. Grimont.** 1987. Identification and biotyping of clinical isolates of *Acinetobacter.* Ann. Inst. Pasteur Microbiol. **138**:569–578.
5. **British Society for Antimicrobial Chemotherapy.** 1991. A guide to sensitivity testing. J. Antimicrob. Chemother. **27**:1–50.
6. **Clark, R. B.** 1996. Imipenem resistance among *Acinetobacter baumannii*: association with reduced expression of a 33–36 kDa outer membrane protein. J. Antimicrob. Chemother. **38**:245–251.
7. **Corbella, X., J. Ariza, C. Ardanuy, M. Vuelta, F. Tubau, M. Sora, M. Pujol, and F. Gudiol.** 1998. Efficacy of sulbactam alone and in combination with ampicillin in nosocomial infections caused by multiresistant *Acinetobacter baumannii.* J. Antimicrob. Chemother. **42**:793–802.
8. **Deutsche Industrie Norm-Medizinsche Mikrobiologie.** 2000. Methoden zur Empfindlichkeitsprüfung von bakteriellen Krankheitserregern gegen Chemotherapeutika. Beiblatt 1 zu DIN 98940–4. Deutsches Institut für Normung e.V., Berlin, Germany.
9. **Gales, A. C., R. N. Jones, K. R. Forward, J. Linares, H. S. Sader, and J. Verhoef.** 2001. Emerging importance of multidrug-resistant Acinetobacter species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the SENTRY Antimicrobial Surveillance Program (1997–1999). Clin. Infect. Dis. **32**:S104–S113.
10. **Grundmann, H. J., K. J. Towner, L. Dijkshoorn, P. GernerSmidt, M. Maher, H. Seifert, and M. Vaneechoutte.** 1997. Multicenter study using standardized protocols and reagents for evaluation of reproducibility of PCR-based fingerprinting of *Acinetobacter* spp. J. Clin. Microbiol. **35**:3071–3077.
11. **Heinemann, B., H. Wisplinghoff, M. Edmond, and H. Seifert.** 2000. Comparative activities of ciprofloxacin, clinafloxacin, gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, and trovafloxacin against epidemiologically defined *Acinetobacter baumannii* strains. Antimicrob. Agents Chemother. **44**:2211–2213.
12. **Henwood, C. J., T. Gatward, M. Warner, D. James, M. W. Stockdale, R. P. Spence, K. J. Towner, D. M. Livermore, and N. Woodford.** 2002. Antibiotic resistance among clinical isolates of Acinetobacter in the UK, and in vitro evaluation of tigecycline (GAR-936). J. Antimicrob. Chemother. **49**:479–487.
13. **Jimenez Mejias, M. E., J. Pachon, B. Becerril, J. Palomino-Nicas, A. Rodriguez-Cobacho, and M. Revuelta.** 1997. Treatment of multidrug-resistant *Acinetobacter baumannii* meningitis with ampicillin/sulbactam. Clin. Infect. Dis. **24**:932–935.
14. **Jones, R. N., and M. N. Dudley.** 1997. Microbiologic and pharmacodynamic principals applied to the antimicrobial susceptibility testing of ampicillin/sulbactam: analysis of the correlations between in vitro test results and clinical response. Diagn. Microbiol. Infect. Dis. **28**:5–18.
15. **Landman, D., J. M. Quale, D. Mayorga, A. Adedeji, K. Vangala, J. Ravishankar, C. Flores, and S. Brooks.** 2002. Citywide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, N.Y.: the preantibiotic era has returned. Arch. Intern. Med. **162**:1515–1520.
16. **Levin, A. S.** 2002. Multiresistant *Acinetobacter* infections: a role for sulbactam combinations in overcoming an emerging worldwide problem. Clin. Microbiol. Infect. **8**:144–153.
17. **Lopez-Hernandez, S., T. Alarcon, and M. Lopez-Brea.** 1998. Carbapenem resistance mediated by beta-lactamases in clinical isolates of *Acinetobacter baumannii* in Spain. Eur. J. Clin. Microbiol. Infect. Dis. **17**:282–285.
18. **Marques, M. B., E. S. Brookings, S. A. Moser, P. B. Sonke, and K. B. Waites.** 1997. Comparative in vitro antimicrobial susceptibilities of nosocomial isolates of *Acinetobacter baumannii* and synergistic activities of nine antimicrobial combinations. Antimicrob. Agents Chemother. **41**:881–885.
19. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard M7–A5. NCCLS, Wayne, Pa.
20. **Pandey, A., A. Kapil, S. Sood, V. Goel, B. Das, and P. Seth.** 1998. In vitro activities of ampicillin-sulbactam and amoxicillin-clavulanic acid against *Acinetobacter baumannii.* J. Clin. Microbiol. **36**:3415–3416.
21. **Rodriguez-Hernandez, M. J., L. Cuberos, C. Pichardo, F. J. Caballero, I. Moreno, M. E. Jimenez-Mejias, A. Garcia-Curiel, and J. Pachon.** 2001. Sulbactam efficacy in experimental models caused by susceptible and intermediate *Acinetobacter baumannii* strains. J. Antimicrob. Chemother. **47**:479–482.
22. **Rodriguez-Hernandez, M. J., J. Pachon, C. Pichardo, L. Cuberos, J. Ibanez-Martinez, A. Garcia-Curiel, F. J. Caballero, I. Moreno, and M. E. Jimenez-Mejias.** 2000. Imipenem, doxycycline, and amikacin in monotherapy and in combination in *Acinetobacter baumannii* experimental pneumonia. J. Antimicrob. Chemother. **45**:493–501.
23. **Seifert, H., A. Schulze, R. Baginski, and G. Pulverer.** 1994. Comparison of four different methods for epidemiologic typing of *Acinetobacter baumannii.* J. Clin. Microbiol. **32**:1816–1819.
24. **Seifert, H., A. Strate, and G. Pulverer.** 1995. Nosocomial bacteremia due to *Acinetobacter baumannii*: clinical features, epidemiology, and predictors of mortality. Medicine **74**:340–349.
25. **Simpson, I., J. Durodie, S. Knott, B. Shea, J. Wilson, and K. Machka.** 1998. Effects of following National Committee for Clinical Laboratory Standards and Deutsche Industrie Norm-Medizinische Mikrobiologie guidelines, country of isolate origin, and site of infection on susceptibility of *Escherichia coli* to amoxicillin-clavulanate (augmentin). J. Clin. Microbiol. **36**:1361–1365.
26. **Sorgel, F., and M. Kinzig.** 1993. The chemistry, pharmacokinetics and tissue distribution of piperacillin/tazobactam. J. Antimicrob. Chemother. **31**:39–60.
27. **Thomson, C. J., R. S. Miles, and S. G. Amyes.** 1995. Susceptibility testing with clavulanic acid: fixed concentration versus fixed ratio. Antimicrob. Agents Chemother. **39**:2591–2592.
28. **Urban, C., E. Go, N. Mariano, and J. J. Rahal.** 1995. Interaction of sulbactam, clavulanic acid and tazobactam with penicillin-binding proteins of imipenem-resistant and imipenem-susceptible *Acinetobacter baumannii.* FEMS Microbiol. Lett. **125**:193–197.
29. **Valcke, Y. J., R. A. Roosel, R. A. Pauwells, M. G. Bogaert, and M. E. van der Straeten.** 1990. Penetration of ampicillin and sulbactam in the lower airways during respiratory infections. Antimicrob. Agents Chemother. **34**:958–961.
30. **Visalli, M. A., M. R. Jacobs, T. D. Moore, F. A. Renzi, and P. C. Appelbaum.** 1997. Activities of beta-lactams against *Acinetobacter* genospecies as determined by agar dilution and E-test MIC methods. Antimicrob. Agents Chemother. **41**:767–770.
31. **Wisplinghoff, H., M. B. Edmond, M. A. Pfaller, R. N. Jones, R. P. Wenzel, and H. Seifert.** 2000. Nosocomial bloodstream infections caused by Acinetobacter species in United States hospitals: clinical features, molecular epidemiology, and antimicrobial susceptibility. Clin. Infect. Dis. **31**:690–697.
32. **Wolff, M., M. L. Joly-Guillou, R. Farinotti, and C. Carbon.** 1999. In vivo efficacies of combinations of beta-lactams, beta-lactamase inhibitors, and rifampin against *Acinetobacter baumannii* in a mouse pneumonia model. Antimicrob. Agents Chemother. **43**:1406–1411.
33. **Wood, G. C., S. D. Hanes, M. A. Croce, T. C. Fabian, and B. A. Boucher.** 2002. Comparison of ampicillin-sulbactam and imipenem-cilastatin for the treatment of *Acinetobacter* ventilator-associated pneumonia. Clin. Infect. Dis. **34**:1425–1430.